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Vaccine for control of fertility

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SUMMARY

Birth control vaccines constitute a new category of vaccines. Immunization with the objective of selectively blocking a physiological process differs in many respects from immunizing against pathogens. Conceptually, these widen the orbit of therapeutic intervention by immunological methods. Success recorded in making vaccines regulating fertility offers models to regulate any other physiological process in the body. At a practical level, the task of making such vaccines is beset with inherent difficulties and with new challenges. This article, dedicated to Avrion Mitchison, aims to discuss these problems and to record successes wherever achieved.

Is it possible to regulate fertility by immunological approaches?

The answer is provided in the affirmative by two sets of observations: (i) the existence of natural infertility in humans (where the reasons can be ascribed to immunological factors), and (ii) the ability to induce infertility by deliberate immunization. The first attempts in this direction were made in 1899 by Landsteiner and Metchnikoff, independently, to produce antibodies agglutinating sperm. Since then much ground has been covered. Defined antigens, and some times submolecular domains of antigens, have been used in place of the whole tissue. Evidence for control of fertility has been obtained by passive and/or active immunization with the following antigens: βhCG (Hearn, 1976; Stevens, 1976; Talwar *et al.*, 1976, 1980), βoLH (Thau, 1988), oFSH (Moudgal *et al.*, 1988), GnRH (Arimura *et al.*, 1973; Fraser *et al.*, 1974; Talwar *et al.*, 1984, 1985), $LDHC_4$ (Goldberg, 1983), sperm antigens identified by monoclonals/polyclonals (Saling, Raines & O'Rand, 1983; Naz *et al.*, 1984; Shaha, Suri & Talwar, 1988) and Zona antigens (Dunbar, 1983; Sacco *et al.*, 1986, Isojima *et al.*, 1986).

Exploitation of autoantigenic potential of sperm antigens

The ontogenesis of sperm begins at puberty in mammals. Several proteins developed at this stage are intrinsically 'foreign' to the immune system. These antigens remain sequestered by a tight barrier which separates immune cells from the compartment where spermatogenesis takes place and the tract through which sperms transit. In the event of break down of this barrier, such as in vasectomy, a large number of patients develop antisperm antibodies. Autoimmune aspermic orchitis is induced by infection or by immunization with sperm antigens provided an adjuvant such as Freund's complete adjuvant (FCA) is employed (Voisin & Toullet, 1969). The necessity in these early studies was for an agent (FCA) labilizing the barrier. Without FCA this can be done by a local injection of Bacillus–Calmette–Guerrin (BCG) as demonstrated by us some years back (Talwar *et al.*, 1979). Animals are rendered aspermic without loss of libido. Leydig cells, which are laid during fetal life and programmed as 'self' to the immune system, are spared and they have normal morphology and function. The method can be employed in a reversible modality. With the restoration of the barrier following elimination of bacilli, fertility is regained (Das, Mustafa & Talwar, 1982; Naz & Talwar, 1986).

BCG can be given in the epididymus to produce aspermatogenesis with normal testosterone levels. The only side-effect of the procedure is local inflammation which lasts from 2 to 15 days in different animals. No fever or behavioural change was noticed in monkeys. Blood chemistry and haematological parameters indicate normalicy of endocrine and metabolic functions. The method is effective in every case. A point unknown at this stage is the consequences of repeat injections.

Quasi-permanent fertility can be imposed by a variant of the above procedure. This approach has been found effective for sterilization of the buffalo (Fig. 1), cow bulls, billies, rams and dogs, and will be available commercially for large scale use. An advantage of this method, in contrast to castration by the Burdizzo castrator, is the retention of libido and making of 'teaser' bulls capable of detecting the buffalo in heat without need of hormonal assays, thus improving the percentage success of artificial insemination with progeny-tested semen.

Females can also be immunized with antigens present on sperm. Many laboratories are engaged in identifying antigens unique to sperm which can impair fertility. These have been cited above. Our group has purified an acrosomal fraction from rat testes which migrates as 24,000 MW protein(s) on SDS–PAGE. Antibodies against these proteins react with membrane-associated components on acrosome of the human sperm. Antibodies with similar reactivity were detected in an infertile female patient. The antigen is constitutional to sperm and emerges at the spermatid stage in the rat testis. Antibodies cause agglutination and prevent penetration of mouse oocyte by mouse sperm (Fig. 2). This particular group of antigens is cross-reactive amongst some species, for instance, rodents, primates and man. Mice passively immunized with rabbit antisera against sperm proteins had markedly reduced fertility (Shaha *et al.*,

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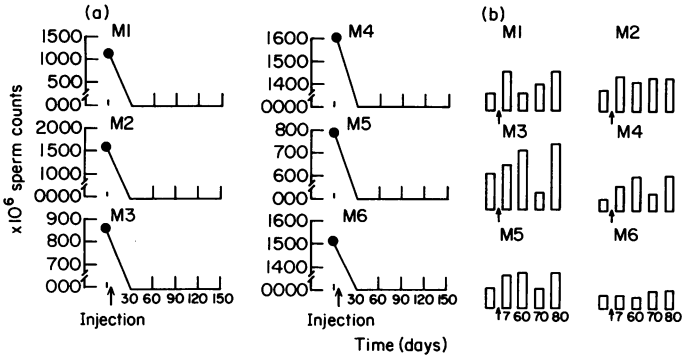

Figure 1. A single injection in cauda epididymis renders bulls azoospermic (a). Random testosterone levels taken at various times after injection have normal levels (b). The bulls retain libido. They mount and donate semen.

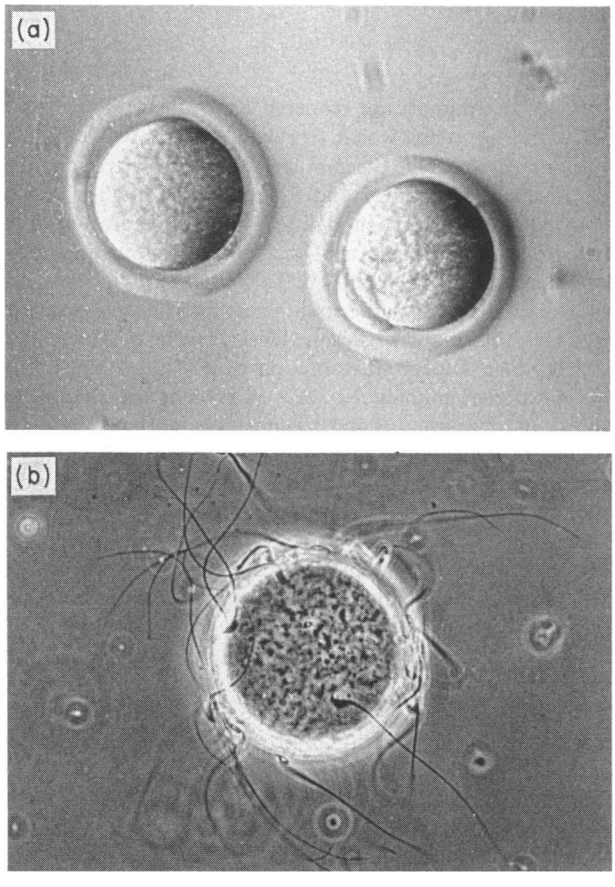

Figure 2. Antibodies against the human sperm acrosmal 40,000 MW fraction (or the immunocross-reactive 24,000 MW in rodents) prevent the binding of mouse sperms to mouse oocytes.

1988). Active immunization of rats leads to drastic reduction of fertility.

Overcoming tolerance

Reproductive processes are heavily dependent on hormones and these have been popular targets of the birth control vaccines. The advantage is that these hormones travel from the producer gland to the target tissue via the blood stream, and antibodies efficient in bioneutralizing hormonal activity can inactivate the hormonal message. The disadvantage in making vaccines against hormones is that they are 'self' components and are not by themselves immunogenic. They require modification by haptens or carriers to induce an immune response. We would like to pay a tribute to Av Mitchison for his pioneering work on immunological tolerance and the ways to break tolerance (Mitchison, 1971a, b; Leech & Mitchison, 1976). Bovine serum albumin (BSA), the conventionally employed carrier in experimental immunology, may not have been appropriate for use in vaccines for humans, partly for fear of cross-reactivity between albumins and mostly for the fact that it is a relatively poor carrier compared with tetanus toxoid (TT) for enhancing immunogenicity (Shastri, Manhar & Talwar, 1981). Vaccines with tetanus toxoid used as carrier have the additional advantage of conferring protective immunity against tetanus.

Genetic restriction of immunological responsiveness to birth control vaccines

Variability of immune response is recognized for most vaccines. A percentage of low responders to vaccines against communicable diseases is acceptable as everyone in the community may not get the infection. However, this is not acceptable in the case of birth control vaccines, as these will be used by women of proven fertility, and inadequate response would not protect them from pregnancy. In our experience, 60% of monkeys immunized with βhCG linked to tetanus toxoid (βhCG-TT) using alum as adjuvant, develop a high response and about 40% of animals have low antibody titres. The percentage of responders can be enhanced by using more potent adjuvants. The use of such adjuvants may, however, bring in other contraindications such as adjuvant arthritis. This may be serious, as such vaccines will be repeatedly injected. We have considered an alternate strategy, to make use of a different carrier to boost antibody titres in monkeys who were hyporesponders to the βhCG-TT vaccine. Figure 3 is illustrative of the benefits of this approach.

Another way to enhance the effectiveness of the fertility control vaccines will be to adopt the *Polyvalent Vaccine* strategy

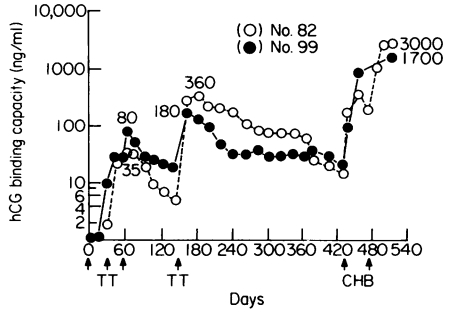

Figure 3. Use of alternate carrier to improve responsiveness in hyporesponders. The change of carrier, cholera toxin chain B(CHB) in place of tetanus toxoid (TT) enhances substantially the antibody titres of monkeys producing relatively low titre with the βhCG-TT vaccine. Representative data from two monkeys.

employing more than one antigen of the reproductive tract, each linked to more than one carrier.

Efficacy

Induction of antibodies against a hormone need not result in effectively abrogating hormonal bioactivity. For instance, an antibody of high RIA titres against thyrotropin (TSH) did not neutralize the TSH bioactivity (Beall *et al.*, 1973). Louvet *et al.* (1974) failed to inactivate hCG bioactivity by sera raised against the 23 amino acid C terminal peptide (CTP) of βhCG, indicating lack of correlation between immunological activity and bioneutralization capacity. For some hormones, such as hCG, the affinity of the hormone for the receptor is high ($\sim 10^{10}$/M). Consequently, antibodies should have an equivalent or higher affinity for binding with the hormone to debar it from acting on the hormone-sensitive tissue. It is for this reason that antibodies induced by the heterospecies dimer, i.e. α oLH-βhCG, were found to have a better bioneutralization potential than those elicited by βhCG (Table 1). The association of βhCG with the αoLH subunit leads to a conformation which recognizes the tissue receptors. The subunits do not have this property. Similar conformation could be generated by the association of homologous subunits (αhCG-BhCG), but the contraindication is the possible cross-reaction with hTSH and hFSH which share the common subunit with hCG. αoLH conserves the association sites with βhCG, but is immunologically non-cross-reactive with human hormones.

Table 1. Comparative bioneutralization capacity of the antibodies generated by βhCG and αoLH-βhCG

Animal species	Immunogen	Bioneutralization potential*
Rat	βhCG-TT (CHB)	62·5+1·5 (6)†
	αoLH-βhCG-TT (CHB)	80·3+2·3 (6)
Bonnet monkeys	βhCG-TT	44·0+3·7 (5)
	αoLH-βhCG-TT	65·2+1·8 (5)

*Neutralization of hCG bioactivity (stimulation of testosterone production by Leyding cells) as a percentage of immunoreactivity (RIA) of antibodies.

†Mean+SEM. Values in parentheses are number of animals immunized.

Specificity

An ideal vaccine would induce immune response by intercepting the desired process with no cross-reaction with other tissues and processes. An argument has persisted on the hazards of cross-reactivity of antibodies generated by βhCG and by its subportion, the carboxyterminal peptide (CTP). βhCG elicits antibodies, with cross-reactivity of 10–50%, with another hormone of the reproductive tract hLH, whereas CTP induce antibodies cross-reactive with pancreatic cells. The degree of cross-reactivity of antibodies with hLH, and their low affinity (Shastri *et al.*, 1978) are perhaps responsible for lack of impairment of ovulation. This cross-reaction is also devoid of adverse pathological effects, as hLH is present in blood and not on the membrane of pituitary cells. Chronic toxicological studies carried out in 63 monkeys hyperimmunized repeatedly for 5–7 years did not demonstrate any pathological abnormalites (Thau *et al.*, 1987; Thau, 1988). On the other hand, quite unexpectedly, the antibodies generated by the CTP vaccine show cross-reaction with pancreatic cells (Rose, Burek & Smith, 1988) presumably because the epitopes of CTP occur on the membranes of these cells. About 60–80% homologies exist in amino acid sequences in as many as 12 human proteins with the two immuno-dominant determinants (Bidart *et al.*, 1987) found in CTP. Use of the whole β subunit of the hormone, where the antibodies are predominantly conformational (and not directed to continuous sequences of amino acids), may in the end have a higher degree of safety.

Current status of clinical trials with vaccines directed against hCG

Three types of hCG vaccines have completed Phase I clinical trials. The vaccine against CTP was tried in 20 women and 10 controls in one centre in Australia. The vaccine against βhCG linked to TT(B) has undergone trials in five centres in India and three centres abroad. Another vaccine in which βhCG was associated with αoLH (A) has also been tried as a parallel formulation in five centres in India. The vaccines based on the entire βhCG(B) or heterospecies dimers(A) were given adsorbed on alum, which is the adjuvant approved for human vaccines. This adjuvant is the only one that has been used on a large scale so far. The CTP vaccine, on the other hand, required the use of Arlacel A, squalane and nor-MDP in a water-in-oil emulsion.

All 90 women, without any exception, receiving B or variant of B vaccine, made antibodies against hCG. Figure 4 gives the response in one of these subjects. In the Australian trial with the CTP vaccine, 12 women out of the initial 20 had little, or no antibodies at all. This was reportedly due to unstable emulsion, and these subjects were replaced subsequently. The antibodies generated by these vaccines have been analysed by Dr Cekan at the Karolinska Institute, employing a common protocol. The antibodies induced by whole βhCG (or its variants) investigated in India were of high affinity ($2{\cdot}5 \times 10^{9}$/M–$3{\cdot}2 \times 10^{10}$M), a component which is not present in the antibodies produced by the CTP vaccine investigated in Australia. Titres were also distinctly higher with our vaccines. In both cases, no other side-effect of

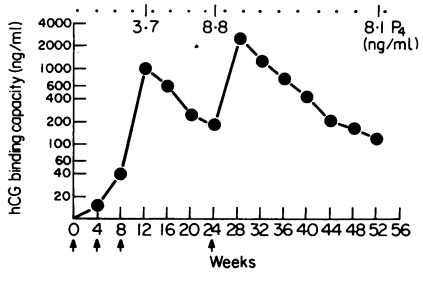

Figure 4. Antibody response in a woman immunized with the vaccine βhCG-TT. For primary immunization, she received three injections at monthly intervals, a booster was given at 24 weeks. Peak titres (hCG binding capacity) after primary immunization were 1000 ng per ml and after booster injection 2890 ng. After one year, the circulating antibody level was 120 ng per ml (20 ng hCG binding capacity is considered adequate to prevent pregnancy). The dots on the upper part of the figure denote the dates of menstruation indicating that cycles were normal and undisturbed by immunization, figures give progesterone values in bleeds at the time indicated, which are indicative of normal ovulatory cycles.

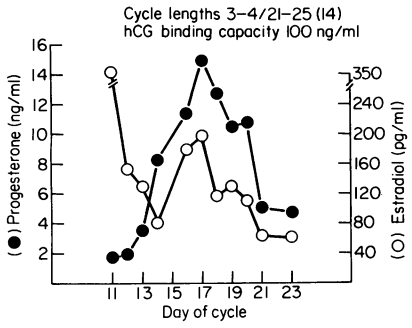

Figure 5. Evidence of normalicy of hormonal profiles in a subject immunized with βhCG-TT. Blood levels of estradiol and progesterone on various days between Days 11 and 23 of the cycle are given. The determinations were made in the cycle when anti-hCG titres were fairly high, e.g. 100 ng/ml (216 ng/ml by the WHO method).

consequence was noted. Menstrual cycles may have been slightly shortened in some women (22% vs. 13% in controls). Bleeds of mid-luteal phase had, however, progesterone values indicative of the normalicy of ovulation. In a few cases, the complete luteal phase was studied by analysis of blood and urinary hormones, and these subjects provide evidence of normal hormonal profiles (Fig. 5).

A puzzling observation in some cases was the suppression of the anti-hCG response at the time of repeated booster injection of βhCG (or variants) linked to TT. These women had recent primary immunization with tetanus prior to induction in the study. This may be similar to the epitope-specific suppression with TT, reported earlier by Herzenberg, Tokuhisa & Hayakawa (1983). Investigations are in progress to determine whether change of carrier or intermittent use of another carrier such as diphtheria toxoid can avert such suppression.

Therapeutic applications of anti-fertility vaccines

Gonadotropin-releasing hormone (GnRH) is a decapeptide common to males and females. Its structure and immunoreactivity is conserved in mouse to man. Several synthetic analogues of GnRH have been made, which interfere in the bioactivity of the hormone. These have found therapeutic applications in hormone-dependent cancers, such as carcinoma of the prostate and breast cancers, and in pathological states such as endometriosis and precocious puberty. GnRH analogues are fairly expensive and need to be administered daily or at frequent intervals. An alternative way to intercept GnRH action is by immunization. A semi-synthetic anti-GnRH vaccine has been made in our laboratory which produces, in a consistent manner, antibodies against this hormone. Immunization causes a marked atrophy of the prostate (Table 2).

Table 2. Effect of immunization of rats with semi-synthetic anti-GnRH vaccine on prostate

Group	Antibody titres (ng/ml)	Prostate weight (mg/100 g body weight)
Control	0	640+28
Immunized	3·67+0·58	36+24

Values represent mean (+SEM) of eight animals.

Looking ahead

Vaccines are indeed feasible for control of fertility. This evidence is, however, at present based on experimental studies in animals, including those conducted in primates. Three vaccines against hCG have reached the stage of clinical testing. Phase I studies completed in India, Australia and other countries demonstrate their ability to induce antibodies against hCG with no notable adverse effect. The efficacy of these vaccines for control of fertility remains to be demonstrated and Phase II efficacy studies would be critical for determining the merits of these vaccines. The vaccine against GnRH may find therapeutic applications in hormone-dependent cancers. One vaccine developed by us inducing aspermatogenesis without loss of libido has reached the stage of commercial production for field use in India.

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